3618-Pos Board B346

Sensing and Stiffening of Tubular Membranes by the Syndapin 1 FBAR

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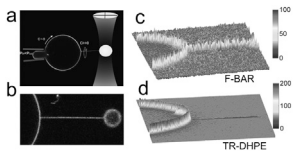
Bin-Amphiphysin-Rvs (BAR) domains are essential components of the cellular machinery responsible for membrane deformation and were found to be sensitive sensors of membrane curvature. One common feature of BAR domains is a curved shape which correlates with high membrane curvatures often found in cells. We focused on characterizing the FBAR domain of Syndapin-1 *in vitro*, given its medical relevance to neurological diseases, using tubular membranes pulled out of Giant Unilamellar Vesicles (GUVs). Specifically, GUVs were synthesized using either porcine brain lipid extracts or the more conventional, binary lipid mixtures to systematically test how lipid composition affects curvature sensing activity. Using optical trapping coupled with force-spectroscopy and confocal microscopy, we discovered an inverse relationship between the curvature sensing activity of the FBAR domain and its equilibrium concentration in solution. At high bulk concentrations of protein, we explicitly measured an increase in the tube's persistence length, which can be understood as mechanical stiffening of the tube. Lastly, we used force spectroscopy to accurately test the effect of the protein on membrane relaxation dynamics in real time.



[1] P.Ramesh, Y.F.Baroji,S.N.S.Reihani, D.Stamou, L.B.Oddershede and P.M. Bendix. Sci. Rep. 3, 1565, 2013.

3619-Pos Board B347

Symmetry and Stability of Membrane Protein Lattices

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Experimental surveys of the protein content in cell membranes suggest that membrane proteins exhibit great diversity in their oligomeric state and transmembrane shape. Furthermore, membrane proteins are often observed to form clusters, which can be composed of thousands of proteins. Recent breakthroughs in high-resolution imaging techniques have shown that the arrangement of proteins in membrane protein clusters is often far from random, with spatial ordering of proteins into regular two-dimensional lattices and, depending on the protein oligomeric state and transmembrane shape, distinct orientational ordering of neighboring membrane proteins. These observations have led, on the one hand, to the proposal that the regular arrangement of membrane proteins may play a role in the biological function of cell membranes. On the other hand, the question arises as to what are the physical mechanisms responsible for the self-assembly, symmetry, and stability of membrane protein lattices. Based on experimental observations and physical models, membrane-mediated interactions between proteins, which result from protein-induced lipid bilayer deformations, have been advanced as a general mechanism for long-ranged interactions between membrane proteins. Here we develop a computational framework for the calculation of membrane-mediated interactions in the large and complicated membrane protein lattices observed in experiments. We find that, depending on the specific shape and oligomeric state of the protein under consideration, membrane-mediated interactions can be attractive or repulsive, several kBT in strength, and depend crucially on the spatial and orientational symmetry in membrane protein lattices. Combining our approach with the theory of regular lattices of polygons, we carry out a systematic survey of the connection between the shape of membrane proteins, and the symmetry and stability of membrane protein lattices. Our results suggest direct experimental tests of how the self-assembly and biological function of membrane protein lattices is influenced by membrane-mediated interactions.

3620-Pos Board B348

Regulation of Neuron Branching by the Interaction of Neuroligin C-Terminus Domain with PIP2

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Intracellular signaling lipids are key regulators of cell physiology. At neuronal synapses and during neuronal development mounting evidence implicates lipids, such as phosphoinositides, as critical regulators. Hence, regulating their cellular distribution is essential for triggering signals in the relevant location. Here we are discussing how neuroligin (nlg-1) contributes to this signaling with its C-terminal domain leading to a thickening of dendrites and the formation of spiny structure in E18 hippocampal neurons as early as DIV4. We have established that overexpression of nlg-1 in HEK293T cells that naturally lack neuron specific proteins, leads to the formation of branches resembling neurite-like protrusions suggesting that early neuroligin expression could contribute to neurite and dendritic arbor formation. We have demonstrated that the morphological changes were linked to local changes in the plasma membrane lipid composition associated to Pi3K and PKC activity. However none of the proteins known to bind to nlg-1 could explain our results. Guided by computational prediction, we tested the relation between nlg-1 and phosphoinositol lipids. We found an increase in PIP2 in membrane expansions in cell expressing nlg-1 wild type compared to nlg-1 C-terminus deletion mutant. Furthermore only cells expressing nlg-1 were affected by a peptide treatment designed to perturb nlg-1 interaction with PIP2. Finally we showed that Nlg1-C-terminus is targeted to the membrane in a PIP2 dependent fashion supporting the hypothesis that nlg-1 harbor an unconventional PDZ domain that interacts with PIP2 to regulate the number of neurite, as well as neurons' branching pattern.

3621-Pos Board B349

Effect of Protein-Induced Spontaneous Curvature on Membrane Surface Tension

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Adsorption of proteins onto membranes can alter the local membrane curvature. This phenomenon has been observed in biological processes such as endocytosis, tubulation and vesiculation. In this letter, we show that the classical elastic model of lipid membranes cannot account for simultaneous changes in shape and membrane tension due to protein adsorption in a local region, and a viscous-elastic formulation is necessary to fully describe the system. Using the viscous-elastic model, we show that protein adsorption can not only induce curvature but also alter membrane surface stress. Using the viscous-elastic model, we show that the lipids flow to accommodate the change in membrane curvature. Finally, at the end of protein adsorption process, the system has a residual stress to balance the difference between the actual mean curvature and imposed spontaneous curvature. Surface stress effects are local and change only in the protein patches, however, curvature changes may be non-local and remain significant for large separations between the protein patches.

3622-Pos Board B350

Oligomerization of H-Ras on Membrane Surfaces

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Ras, a lipid-anchored small molecule GTPase, is an important signaling node in mammalian cells. Ras signaling is regulated by the dynamic nature of its subcellular localization, lateral partitioning and hierarchies of lipid and protein interactions. We studied the clustering of H-Ras on membrane surfaces using a combination of two-color fluorescence correlation spectroscopy (FCS), photon counting histogram (PCH) spectroscopy, time-resolved anisotropy, and single molecule (SM) tracking on reconstituted supported bilayer systems. Various constructs that allow us to tether H-Ras to the membrane at the native lipidation sites were generated. Diffusion behavior of H-Ras on membrane surfaces, both translational and rotational, was measured at a wide range of surface densities. Degree of oligomerization of H-Ras was quantified by PCH and verified by SM tracking. The dissociation constants of the two nucleotide states of Ras were determined. In addition, a mutant of H-Ras, which exhibited different clustering behavior on membrane surfaces was compared against wild type H-Ras. The effect of membrane surfaces on Ras oligomerization and the sensitivity of the oligomerization to membrane anchor structure will be discussed.